Transcranial Doppler Ultrasound in Pediatric Neurocritical Care: Clinical and Research Applications

Genetic Markers Associated with Cerebral Vasospasm in Pediatric TBI

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The content of this presentation does not contain reference to, nor advocates use of, unlicensed medicines or devices.
Objectives

- The use of TCD in pediatric TBI research.
- Study of genetic markers and their relationship to cerebral vasospasm in children with TBI.
Traumatic Brain Injury (TBI)

- 1/3 of all injury-related deaths in the U.S.
- *Leading cause* of morbidity & mortality in children & youth
- Mortality highest among the young, boys, & minorities
- Personal, financial, & societal costs of TBI are immense
- Limited therapeutic interventions currently available make this an area of compelling clinical need
- Diagnostic genetic markers may clinically identify those at risk for poor outcomes
Cerebral Vasospasm (CV) is a secondary injury phenomenon. It leads to cerebral hypoperfusion, ischemia, and vascular insults.

Children with mod-severe TBI found to experience CV by O’Brien, Reuter-Rice et al. 2010; O’Brien et al., 2015.

The prevalence of CV in pediatric TBI is 21% (anterior circulation) and 12% (posterior circulation) by O’Brien et al., 2015.

CV following TBI:
- Time of onset & duration
- Poor neuro-functional outcomes
Study Participant Criteria

**INCLUSION:**
- Previously healthy
- 5 days to 15 years
- Admitted to DUHS with a TBI
- Under standard of care treatment
- GCS 3 to 15
- Capable of adequate TCD ultrasound

**EXCLUSION:**
- Presence of previous neurodevelopmental delay
- Diagnosis of non-traumatic intracranial hemorrhage

![Image of study participants]
Study Design

- 3 year prospective exploratory study (2013-2015)
- \( N = 60 \) children admitted for TBI to DUHS
  - Presence of CV was determined by using Transcranial Doppler ultrasound (Sonara Digital TCD, Natus)
Biologic Analyses

- Genotyping by TaqMan® allele discrimination assays for the presence of genetic markers
  - Genetic markers (Apolipoprotein E [APOE], Endothelin 1 [EDN1])

- Additional protein analyses performed by Astute Medical Inc, San Diego CA
  - Neuroinflammatory associated biomarkers: GFAP, CKBB, NSE, S100B, CRP, IL6, ET-1
TCD Timing and Measures

- **TCD within 24hrs of admission for CBFV & LR** Aaslid et al. 1982; Bode & Wais, 1988; O’Brien, 2015

<table>
<thead>
<tr>
<th>High MCA flow velocity</th>
<th>( V_{mca} )</th>
<th>( \geq 2 \text{ SD above normal age value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV ((V_{mca} + LR))</td>
<td>( V_{mca} )</td>
<td>( \geq 2 \text{ SD above normal age value} )</td>
</tr>
<tr>
<td></td>
<td>LR</td>
<td>( V_{mca} : V_{EC-ICA} \geq 3 )</td>
</tr>
<tr>
<td>BA vasospasm</td>
<td>( V_{ba} )</td>
<td>( \geq 2 \text{ SD above normal age value} )</td>
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</table>


- **Glasgow Outcome Scale-Extended Pediatrics** Beers et al. 2012
  - *at discharge (T1) and 4-6 weeks post discharge (T2)*
### Parent Study Sample Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results (N=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission location, (n) %</td>
<td></td>
</tr>
<tr>
<td>Pediatric ICU</td>
<td>(40) 67</td>
</tr>
<tr>
<td>Non-ICU</td>
<td>(20) 33</td>
</tr>
<tr>
<td>Gender, (n) %</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(34) 57</td>
</tr>
<tr>
<td>Female</td>
<td>(26) 43</td>
</tr>
<tr>
<td>Mean Age, years</td>
<td>5.5</td>
</tr>
<tr>
<td>Race, (n) %</td>
<td></td>
</tr>
<tr>
<td>African American/ Black</td>
<td>(20) 33</td>
</tr>
<tr>
<td>Caucasian/White</td>
<td>(37) 62</td>
</tr>
<tr>
<td>Other</td>
<td>(3) 5</td>
</tr>
<tr>
<td>Ethnicity, (n) %</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic or Latino</td>
<td>(53) 88</td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>(7) 12</td>
</tr>
<tr>
<td>Glasgow Coma Scale (GCS), (n) %</td>
<td></td>
</tr>
<tr>
<td>Mild (GCS 13-15)</td>
<td>(44) 73</td>
</tr>
<tr>
<td>Moderate (GCS 9-12)</td>
<td>(2) 3</td>
</tr>
<tr>
<td>Severe (GCS 3-8)</td>
<td>(14) 24</td>
</tr>
<tr>
<td>Mechanism of Injury, (n) %</td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>(23) 39</td>
</tr>
<tr>
<td>Abusive Head Trauma</td>
<td>(18) 30</td>
</tr>
<tr>
<td>Motor vehicle related</td>
<td>(7) 11</td>
</tr>
<tr>
<td>Other/recreational</td>
<td>(12) 20</td>
</tr>
<tr>
<td>Diagnosed Injury, (n) %</td>
<td></td>
</tr>
<tr>
<td>Subdural</td>
<td>(27) 45</td>
</tr>
<tr>
<td>Epidural</td>
<td>(13) 22</td>
</tr>
<tr>
<td>Subarachnoid</td>
<td>(13) 22</td>
</tr>
<tr>
<td>Other</td>
<td>(7) 11</td>
</tr>
<tr>
<td>Mean Length of Stay, days</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Represents expected population:

- Boys > girls
- 38% non-white
- Strong representation of mild & severe
- Majority falls
- High # subdural injury
- Mean LOS ~10 d
Study #1:
The Effect of the Relationship of APOE Polymorphisms and Cerebral Vasospasm on Functional Outcomes in Children with Traumatic Brain Injury

Karin Reuter-Rice, PhD, NP, Michael Regier, PhD, Ellen Bennett, PhD, & Daniel Laskowitz, MD, MS

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Apolipoprotein E (APOE)

- **APOE** is a protein coding gene
- 3 common protein isoforms: **APOE2**, **APOE3**, **APOE4**

- SNP rs405509, results in a G/T nucleotide change at position -219 and modifies APOE gene expression. Bennett, Reuter-Rice, Laskowitz, 2016

- A study of 185 adult with SAH found the presence of the APOE4 allele was a risk factor for CV. Wu et al. 2010
Apolipoprotein E ($APOE$) has been linked to CV and poor outcomes in adults with TBI (Lee et al. 1997; Oertel et al. 2005).


No pediatric TBI studies examining $APOE4$ SNPs and CV

**AIM:** Examine the relationship between the $APOE$, specifically $APOE4$ SNPs (rs405509, rs429358, rs7412) and CV to neuro-functional outcomes in children with TBI.
CV Incidence & Genotypes

- CV incidence 43.3% (n=26)
  - Anterior circulation: n=5 vs. Posterior circulation: n=25
  - Males 54% vs. Females 46%
  - Most prevalent with hx of falls (42%)
  - Most prevalent in young <6yo (65%)

- APOE genotype
  
<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>No CV</th>
<th>Yes CV</th>
<th>p=0.649</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2E3</td>
<td>3 (8.8)</td>
<td>5 (19.2)</td>
<td></td>
</tr>
<tr>
<td>E3E3</td>
<td>23 (67.7)</td>
<td>16 (61.5)</td>
<td></td>
</tr>
<tr>
<td>E3E4</td>
<td>7 (20.6)</td>
<td>5 (19.2)</td>
<td></td>
</tr>
<tr>
<td>E4E4</td>
<td>1 (2.9)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>
There were significant differences in injury mechanism (unadjusted \( p = 0.048 \)) and age (unadjusted \( p = 0.02 \)) between those with and without CV.

The noncoding promoter SNP rs405509 T/T, when considered with injury severity, appeared to modify the relationship of APOE genotype to CV.

The relationship between APOE and CV had no significant effect on GOS-E Peds scores.

Higher incidence of CV in posterior circulation.
Study #2:

Endothelin 1 Gene Variant May Play a Role in Cerebral Vasospasm in Children with Traumatic Brain Injury

Karin Reuter-Rice, PhD, NP, Michael Regier, PhD, Ellen Bennett, PhD, & Daniel Laskowitz, MD, MS

Funding:
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Robert Wood Johnson Foundation #71244 (2013-16)
Endothelin 1 (*EDN1*)

- Chromosome position: 6p24.1
- *EDN1* gene encodes ET-1 protein, a potent vasoconstrictor
- rs2070699 (G>T or C) single nucleotide polymorphism (SNP)
- rs5370 (G>T) confers functional change in EDN1 = Lys198Asn
- Associated with CV in SAH  Gallek, 2008
**EDN1 & CV**

- Animal model TBI (fluid percussion injury): changes in cerebral hemodynamics associated with neuronal damage

- Model implicated *EDN1* as a possible role for CV:
  - ET-1 $\rightarrow$ Intracellular Ca$^{++}$ $\rightarrow$ calpain $\rightarrow$ CV
  - Armstead & Raghupathi, 2011

- Genetic variation in *EDN1* may account for the variance observed in functional outcomes in adult SAH Arutjunov et al. 1975; McGirt et al. 2002 Siman et al. 2011

- Trend found between *EDN1* SNP rs6912834 & angiographic vasospasm
**EDN1 & CV**

- Children with severe TBI showed ↑ levels of ET-1 over time
  - Associated with poor GOS & functional outcomes
  - Patients had sustained vasoconstriction & ↓ cerebral blood flow *suggestive of vasospasm* (TCD not measured) Salonia et al. 2010

No pediatric TBI studies examining *EDN1* SNPs and CV

**AIM:** *EDN1* rs5370, rs2070699 association with CV in pediatric TBI
CV Incidence by Circulation, Injury Severity, & Lesion

- Overall CV 43% incidence (n=26)
  - Anterior circulation (n=5)

- Posterior circulation: (n=25)
  - Children with *mild* TBI (all ages) had highest incidence

- Subdural hemorrhage associated with the highest incidence (52%)
EDN1 SNP Study Findings

- Of the 60 children, 85% \( (n=51) \) had genotype data \( (n=9 \text{ missing data}) \)

- Children with any copy of the rs2070699 (T) risk allele (60%) showed trend towards greater incidence of CV compared to G/G homozygous carriers \( (p=0.07) \)

- No significant difference in rs5370 (T) risk allele carrier status (40%) between CV positive and CV negative children \( (p=1.0) \)
Genetic Implications in Pediatric TBI Research

- APOE4 SNP rs405509 *may modify* the relationship between APOE and CV in children with TBI
  - More studies are needed; examine the age effect of APOE
- First to report evidence of *gene association trend* with presence of *EDN1 SNP rs2070699 risk allele (T)* and *incidence of CV* among children with TBI
  - More studies to compare these findings in adults with TBI
- Candidate *EDN1* and *APOE4* demonstrate limited evidence  
  - Reuter-Rice et al. 2018
- Novel marker discovery needed for future powered research
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**Co-Is:** E. Bennett, PhD, D. Laskowitz, MD

**Stats:** M. Regier, PhD

DUHS Peds Critical Care and Neurosurgery

TCD sonographers

Pediatric Speech Pathology

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**Nurse Champions:** Ron, Ryan, Martina, Erin, May

Duke University, Brain Injury Translational Research Center

(Lab): Bennett, Laskowitz, etc.